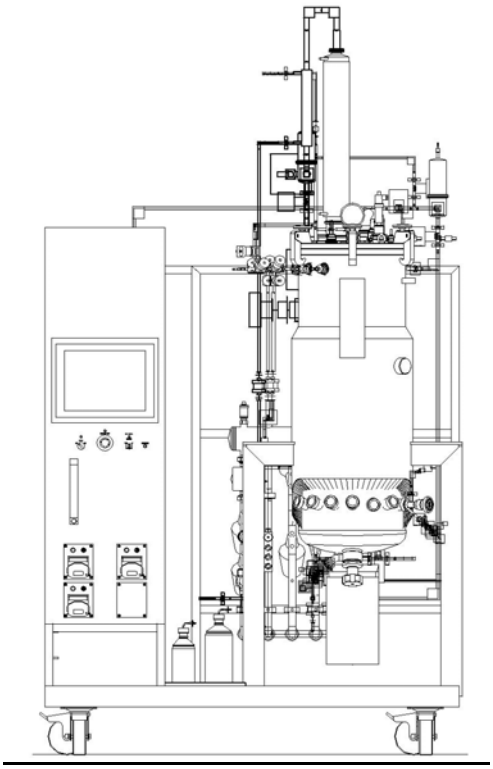
i-Control
for
In Situ sterilizable 140L Bioreactor



HARDWARE MANUAL

Project 48560 (140L Pilot System), UC Davis

June 2009

applikon® BIOTECHNOLOGY

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1 GENERAL SAFETY

1.1 SYMBOLS

The following symbols are used on the equipment and in this manual.

WARNING
Risk of personal injury or danger to life.

WARNING
Risk of electrical shock hazard.

WARNING
Risk of physical hand injury

WARNING
Risk of physical hand injury.

CAUTION
Risk of damage to the equipment.

Additional information

1.2 SAFETY CLASS I EQUIPMENT

GENERAL

- This set of equipment has been designed in accordance with EN61010-1 "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use", and has been supplied in a safe condition.
- The Hardware manual contains information and warnings, which have to be followed by the user to ensure safe installation, operation and to retain the equipment in safe condition.
- Before switching on the equipment, make sure that it is set to the line voltage.
- This equipment is designed for bioprocess control; it must not be used for other purposes!

WARNING
Risk of electrical shock hazard.

- Any interruption of the protective conductor inside or outside the apparatus or disconnection of the protective conductor terminal will make the apparatus dangerous. Intentional interruption is prohibited.
- Capacitors inside the apparatus may still be charged, even if the apparatus has been disconnected from all voltage sources.
- Any adjustment, maintenance and repair of the opened apparatus under voltage must be avoided as far as possible and, if inevitable, the activities must only be carried out by qualified personnel.
- Make sure that only fuses with the required rated current and of the specified type (IEC 127) are used for replacement. The use of makeshift fuses and the short-circuiting of fuse holders are prohibited.
- It is not permitted to connect equipment to the ***i-Control*** or the bioreactor without the qualification of double isolation or without the SELV (Separated Extra Low Voltage) qualification.

WARNING
Risk of physical hand injury caused by pumps.

- In most cases, one or more pumps are installed in the ***i-Control***.
- According to the European Community legislation on machinery, a pump drive is a machine with potential hazard. Only apply these drives with the pump heads and tubing that are described in this manual (chapter: pumps)!
- Switch off the power of the pump drive before replacing any tubes or pump heads.

CAUTION
Risk of damage to the equipment.

- Do not use the tubing pump drives for other purposes than displacement of fluids (or gas).
- Application of damaged tubes may result in fluid leaking into the pump drive.
- Verify the tube quality inside the pump head before every fermentation run.

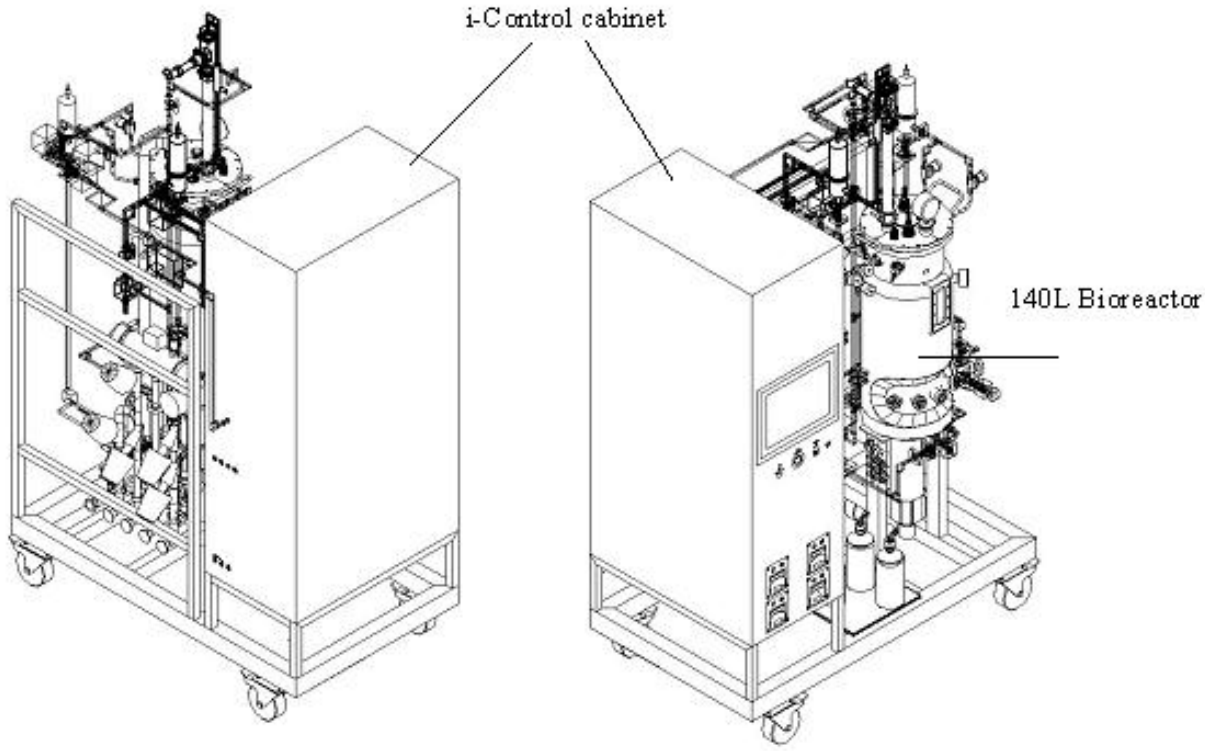
Additional information

- Do not apply an Earth Leakage Circuit Breaker in the power supply of the Applikon control equipment (in an industrial environment or laboratory, an earth leakage circuit breaker is not mandatory).
- Because of the built-in main supply filters, an Earth Leakage Circuit Breaker may be activated. As a result, the power will be switched off!
- Additional electrical protection can be realized by installing a separation transformer between the mains and the control equipment.

2 140L PILOT SYSTEM

The 140L Pilot System consist of the following parts:

- An In Situ sterilizable Bioreactor with the appropriate auxiliaries, like a stirrer assembly, baffles, sensors, an aeration assembly, etc.
- A wet part for temperature control and steam sterilization.
- A Bioconsole (the *i*-Control) for measurement and control of process variables (pH, temperature, DO, level, pressure, and stirrer speed) with corresponding controller outputs in order to keep process conditions at set-point.
- The isometric representation (given below) shows the main components of the 140L Pilot System on a skid



3 THE *i*-CONTROL

The *i*-Control combines and supports actuators like pumps and valves in order to optimize the use of limited bench space. A survey of the *i*-Control for the 140L bioreactor is presented in the following sections.

3.1 VIEWS

3.1.1 FRONT AND REAR VIEW

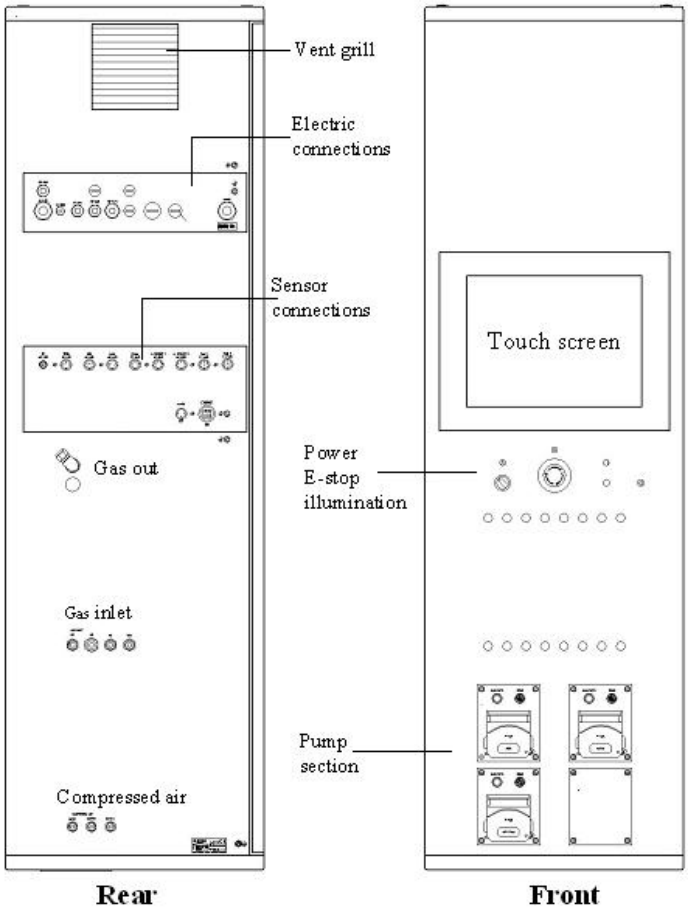


Figure: The front and rear exterior of the *i*-Control cabinet:

3.1.2 BOTTOM VIEW

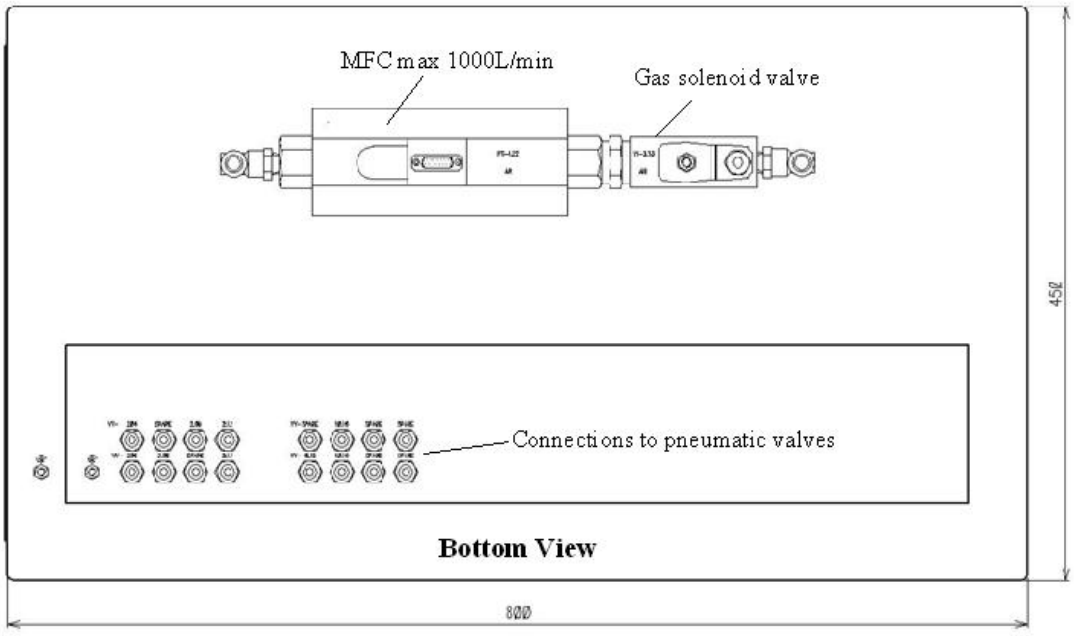


Figure: Bottom view. Note the MFC max 1000L/min is located on the bottom panel while the remaining MFCs are on the inside

3.1.3 INSIDE LEFT VIEW

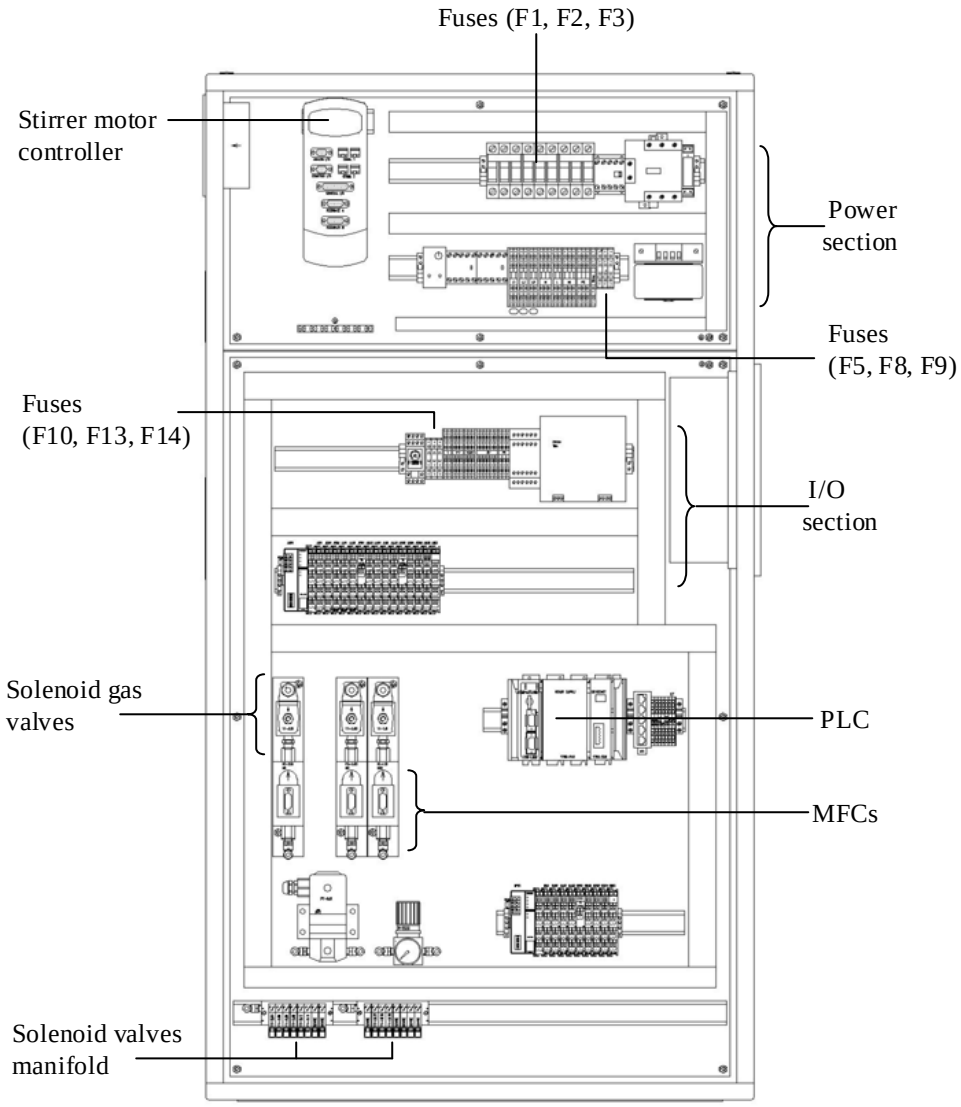


Figure: In side left view with door open

3.1.4 FUSE SPECIFICATIONS

Fuse number	**Application**	**Type**	**Rating**
Fuse F1	Main fuses (×3)	Ceramic 10 ×38 mm	20A
Fuse F2	Heating element (×3)	Ceramic 10 ×38 mm	12A
Fuse F3	Stirrer motor (×3)	Ceramic 10 ×38 mm	12A
Fuse F5	Power supply	5 ×20 mm	T 8A
Fuse F8	Circulation pump	5 ×20 mm	T 4A
Fuse F9	Illumination	5 ×20 mm	T 2A
Fuse F10	Temperature Switch	5 ×20 mm	T 2A
Fuse F13	Central Alarm	5 ×20 mm	T 1A
Fuse F14	Fan	5 ×20 mm	T 250mA

3.1.5 ELECTIRICAL CONNECTIONS

Electrical connections for the mains, illumination are located at the rear exterior of the *i*-Control cabinet.

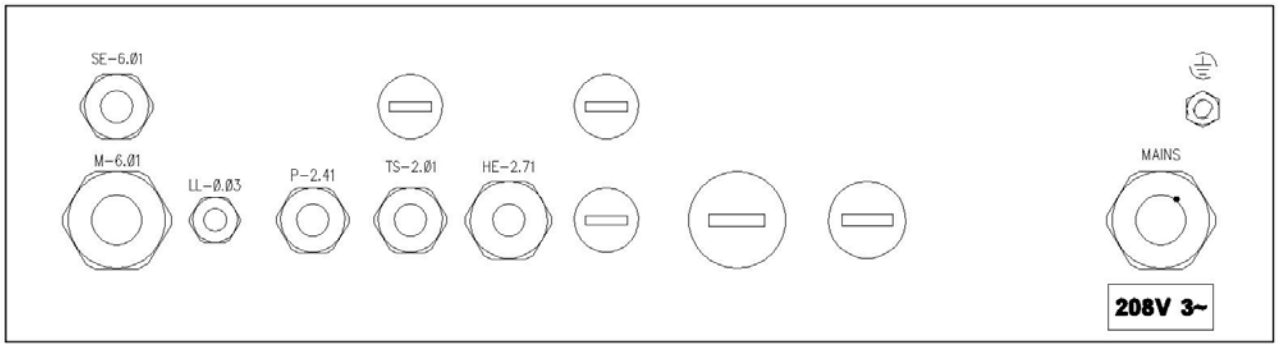


Figure: Electrical connections

The following electrical connections are present (also shown in the figure)

- SE-6.01: Encorder cable connection
- M-601: Motor cable connection
- LL-0.03: Connection for illumination in the reactor
- P-2.41: Connection for the circulation pump
- TS-2.01: Temperature Switch connection
- HE-2.71: Connection for the wet part heating element
- Main: Main Entry (208V, 3~) connection

3.1.6 SENSOR & I/O CONNECTIONS

Sensor and I/O connections are externally located on the rear side of the i-Control cabinet. The following sensor connections are present (also shown in the figure)

- BNC connection for the pH sensor
- 3-pin audio connection for the DO sensor
- 3-pin audio connection for the Temperature sensor
- 2-pin audio connection for the Level sensor
- 5-pin audio connection for pressure sensor
- Two spare 5-pin audio connection for analog sensors
- Two spare 5-pin audio connection for feed pumps (P-14.01 and P-14.02)

Below the sensor connections, two I/O connections are located:

- 2-pin audio connector for the alarm
- RJ45 Ethernet connection:
  - Supervisory Control And Data Acquisition (SCADA) program on a Host
  - Remote access for remote control by a user

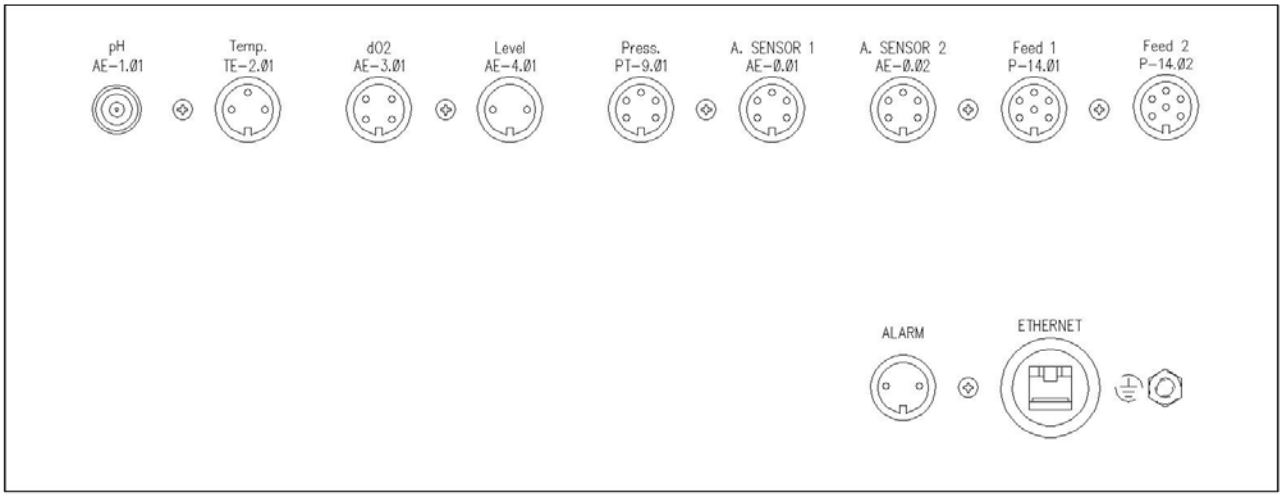


Figure: Sensor & I/O connections

3.1.7 PNEUMATIC I/O CONNECTIONS

Bulkhead connections for compressed air inlet and outlet and for actuation of the Back Pressure Control Valve (B.P.C.V.) are present at the exterior of the rear panel of the *i*-Control cabinet.

Bulkhead connector size: OD 6 mm air tubing.
Required air inlet pressure: 4 - 6 barg.

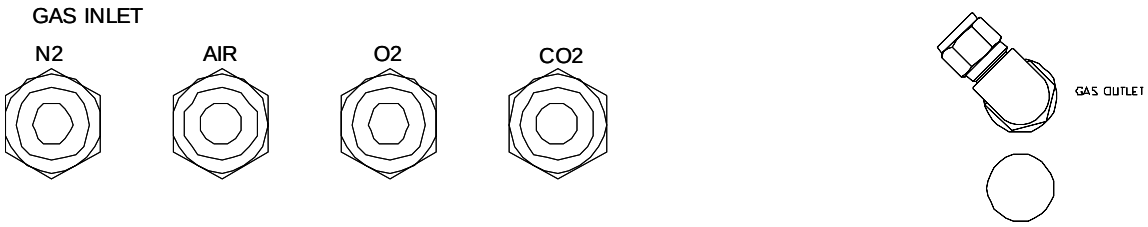
Connections to the pneumatic valves in the system are located at the bottom side of the cabinet

The table below lists the pneumatic I/O connections and their functions.

V-2.04	Circulation Valve
V-2.06	(Clean) Steam Inlet Valve
V-2.08	Circulation Valve
V-2.09	Water Return Valve
V-2.12	Drain Valve
V-2.13	Main Steam Inlet Valve
V-10.15	Steam to Inlet Filter Valve
V-10.16	Condensate Valve (Inlet Filter to Biowaste)
V-10.19	Gas Inlet Valve

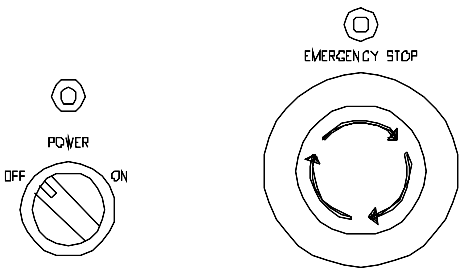
3.1.8 GAS INLETS AND OUTLET

The gas inlet connections for carbon dioxide, oxygen, and nitrogen are Quick Connectors for tubing OD = 10 mm. The inlet connection for the externally located air MFC max 1000L/min is a 12 mm pipe. Required inlet pressure for the gasses: 2 barg. The gas inlet connections are located at the rear panel of the *i*–Control cabinet. One bulkhead connection for gas out is present just below the electrical connections on the rear panel.



3.1.9 MAINS CONNECTION AND POWER SECTION

The power section is located at the front of the *i*-Control (mains switch and Emergency Stop button):



The power of the Bio Console is switched on by turning the power switch 90° clockwise.

MAINS SUPPLY: 208V 3~ High Voltage Current
CAUTION! ELECTRICAL SHOCK HAZARD!
Connect the main supply cable to the power source according to:

- brown (bn) = L1,
- black (bk) = L2,
- grey (gy) = L3,
- blue (bu) = neutral,
- green/yellow (gn/ye) = earth

The *i*-Control is switched on by turning the power switch clockwise.

Caution:
Minimum specification of the mains cable = 5 x 4.0 mm^2. The *i*-Control cabinet must ONLY be serviced by **qualified personnel**. During service, the *i*-Control must be powered down. In order to switch off the *i*-Control completely for service purposes, first switch off power supply to the cabinet then unplug the Mains Cable supplying power to the cabinet.
Using an earth of poor quality may cause malfunctioning of the control equipment!

Pushing the Emergency Stop mushroom button powers down all actuators. The controller continues measuring process variables, but process control is disabled during E-stop intervals.

Reset the Emergency Stop status as follows

Press the function key Unit Layer, followed by the key Power ON System.

The I/O hardware is now powered up again.
.

When the illumination button is pushed, the inside of the reactor is illuminated for approx. 1 minute.
Note: The timer range can be adjusted, by qualified persons, in the range 0.05 sec. - 1 hour

3.2 ENVIRONMENTAL CONDITIONS

The *i*-Control cabinet may be used at locations with the following environmental conditions:
- Indoor use,
- Altitude: up to 2000 m,
- Temperature: 4°C to 45°C,
- Maximum relative humidity 80% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 45°C,
- Mains supply voltage: 208V 3~ High Voltage Current
- Transient overvoltages according to INSTALLATION CATEGORIES II,
- POLLUTION DEGREE 2 in accordance with IEC 664,
- P-max = 6900 VA.

3.3 CLEANING INSTRUCTIONS

The ***i***-Control cabinet may be cleaned with a moist tissue in combination with a non-abrasive cleaner.

4 ACTUATORS

The ***i***-Control supports the following actuators:

- pH control: acid / base addition or aeration with carbon dioxide
- DO control: aeration with air, nitrogen or oxygen, stirrer speed control
- Temperature control: the wet part contains a heater tank for temperature control during cultivation and steam valves for In Situ sterilization
- Level control: Level / Foam pump

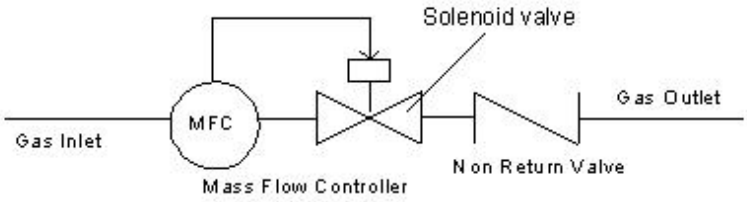
This chapter describes the applicable hardware (inside the ***i***-Control cabinet and outside the bioreactor).

4.1 GAS SUPPLY SECTION

The gas supply section supports up to four mass flow controllers (MFCs). Three MFCs for for N2, O2 and CO2 are located inside the cabinet. The fourth MFC for air is located at bottom of the cabinet. Refer to section 3.1 for location of the mass flow controllers. The mass flow controllers require a gas inlet pressure of 2 barg

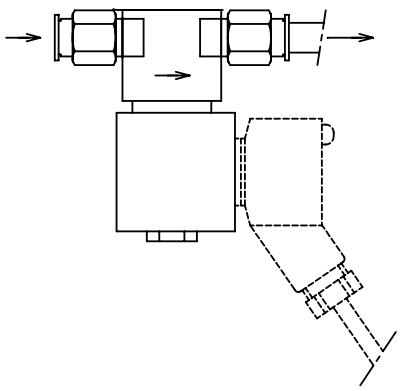
V0R2551100	Mass Flow Controller (×3)	max 100SL/min
V0R2551910	Mass Flow Controller (×1)	max 1000SL/min

Mass flow controllers are configured as shown:



A non return valve prevents pressure differences to cause backflow through the MFC.

A solenoid valve with inhibit function is always mounted in combination with a mass flow controller. A digital output control signal is used to close the solenoid valve in case the controller output reaches its lower limit.



V0R5029312	Solenoid Valve (×3)	24 VDC
V0R5029420	Solenoid Valve (×1)	24 VDC

4.2 ACTUATORS FOR TEMPERATURE CONTROL

The wet part of the system contains actuators for temperature control during cultivation and sterilization. During cultivation, water is circulated through the jacket of the reactor. The temperature in the reactor is controlled by either electrical heating or introducing cold water into the system. During sterilization, steam is introduced into the jacket of the reactor. When the temperature in the reactor exceeds a pre-programmed level, steam is also injected in the reactor through the gas inlet filter.

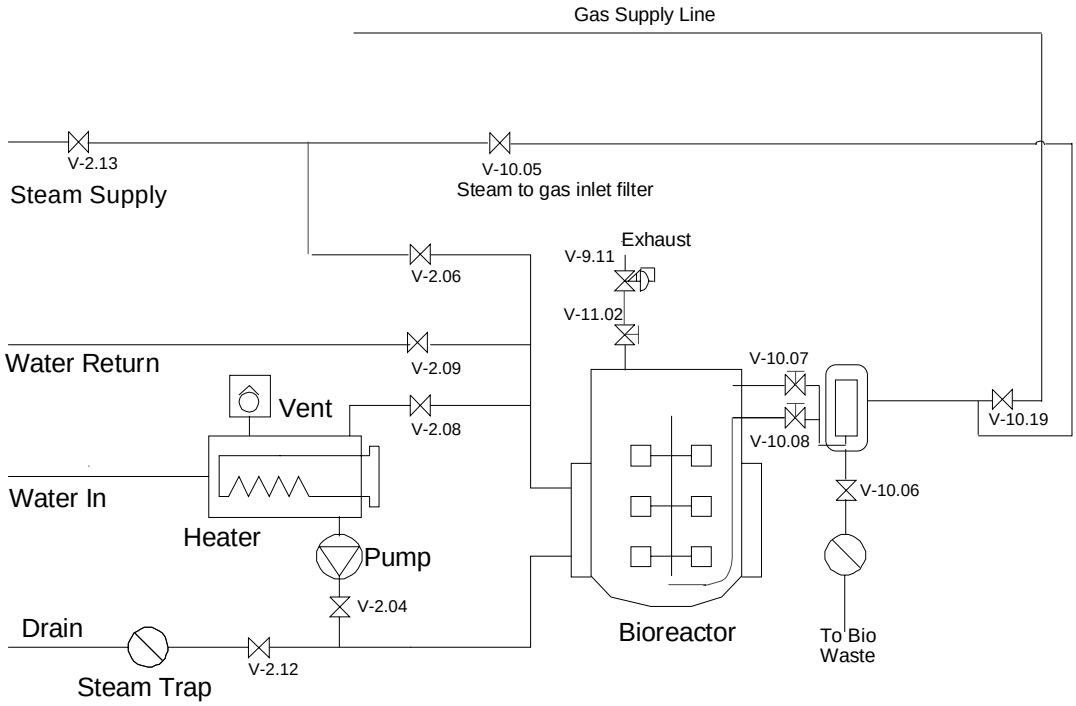
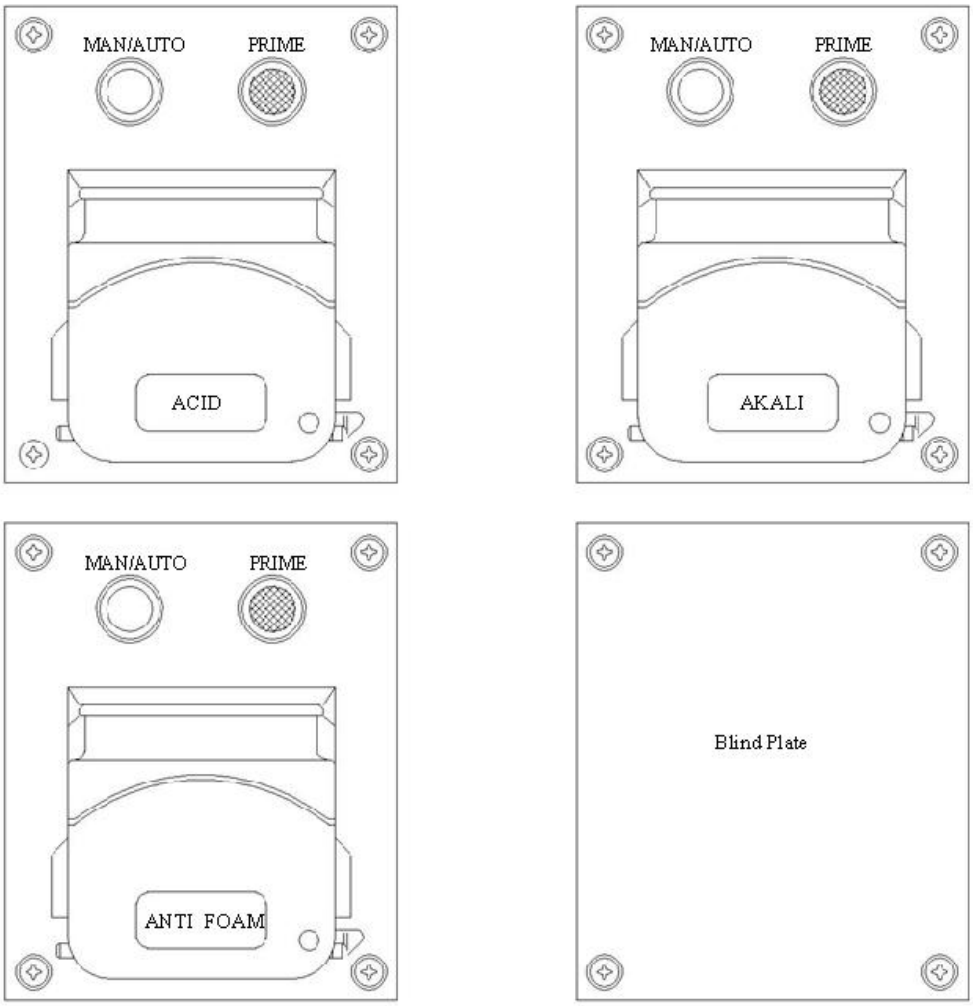


Figure: Schematic layout of the wet part and gas in and outlet lines

4.3 PUMPS AND TUBING

Three pumps (ACID, BASE ANTIFOAM) are installed at the pump section as shown the in the figure below.



Fixed-speed pump drives for ***i***-Control:

V1LH127382 Tubing Pump Watson drive 20 rpm (×3)

External variable speed pumps (with 120V power supply):

Z375548500 MasterFlex drive 100rpm (×2)

For specific information concerning the External variable speed pumps and pump heads, refer to the Masterflex User Manuals that come with the pumps.

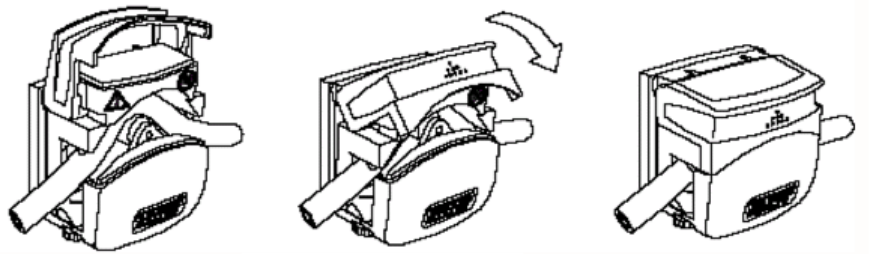
CAUTION

- According to the European Community legislation on machinery, a pump drive is a machine with potential hazard. Only apply these drives with the pump heads and tubing that are described in this chapter.
- Do not use the tubing pump drives for other purposes than displacement of fluids (or gas).
- Switch off the power of the pump drive before replacing any tubes or pump heads.
- Verify the tube quality inside the pump head before every fermentation run.
- Application of damaged tubes may result in fluid leaking into the pump drive.

The Correct way to mount pump tubing

WARNING
Risk of physical injury while loading a pump tubing
Start the pump head manually. Carefully load the tubing in the pump head: **watch your fingers!**

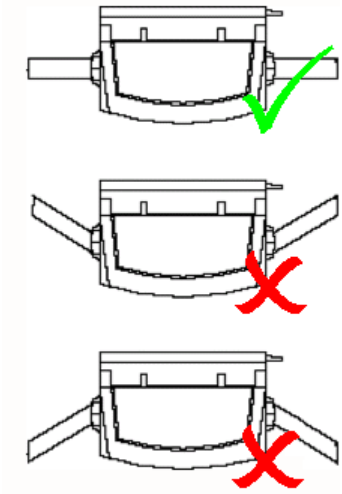
Pump head type 313D (advanced)



Mounting the tube

Tube bend too sharp upwards | Tube bend too sharp downwards | Tube bend correctly

Front view of the pump



Tube not bend

Tube bend too sharp backwards

Tube bend too sharp forewards

Top view of the pump

5 140L BIOREACTOR

The main components (vessel, jacket, tubing, frame) of the Applikon 140L bioreactor are made of SS 316L (1.4404) stainless steel. The sight glass is made of borosilicate, and the gaskets and O-rings are made of silicone, EPDM or PTFE. The rectangular sight glass in the vessel sidewall is mounted at working volume level. The minimum working volume of a reactor is determined by the location of the sensor ports.

Design code and parameters
The reactor is constructed in accordance with the codes:

- ASME - American Society of Mechanical Engineers (section VIII, division 1)

Finishing
The internal reactor surface and the auxiliaries that are mounted inside the reactor are electrolytically polished to achieve a surface roughness of $R_a \leq 0.4$ µm. The inner surfaces are free of crevices to facilitate cleaning and to avoid contamination. The external reactor surface is mirror polished.

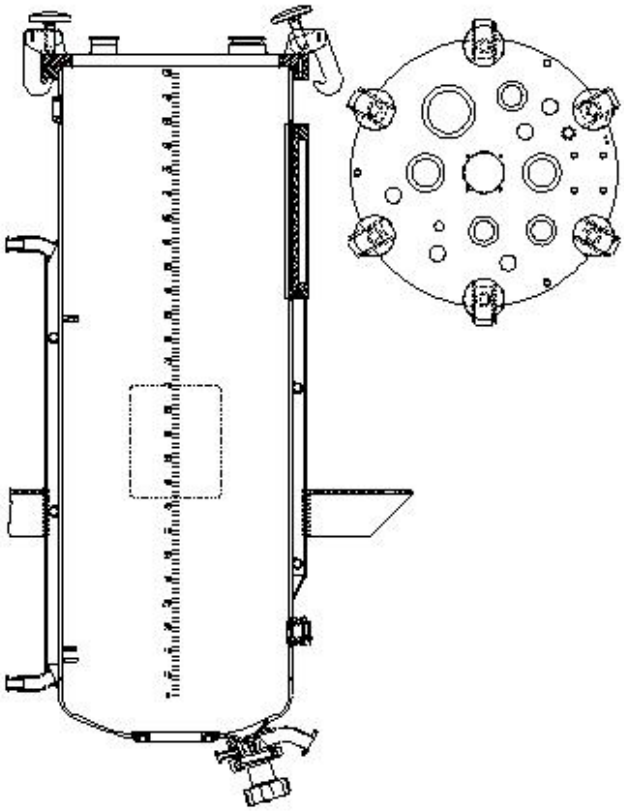
Specifications

All insitu sterilizable bioreactors are designed to the following

- Design pressure reactor 4.1 bar
- Test pressure reactor 4.8 bar
- Design pressure jacket 6.2 bar
- Test pressure jacket 11.2 bar
- Maximum temperature reactor 130 °C

Dimensions

Total volume	140 liter
Working volume	102 liter
Minimum working volume	22 liter
Inner diameter	395 mm
Inner height (maximum)	1158 mm
Liquid height (working volume)	850 mm
H/D total	3.0
H/D working volume	2.2
Floor space (W x D)	1600 x 1000 mm
Total system Height (H)	2900 mm



6 AUXILIARIES

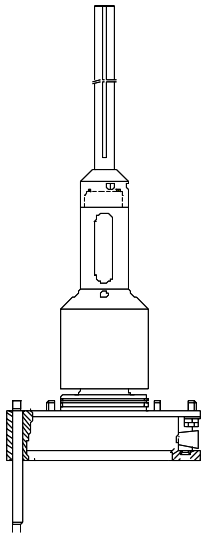
6.1 AGITATION

The bioreactor is provided with a stirring system consisting of:

- a bottom stirrer assembly
- a stirrer motor
- a digital stirrer speed controller
- power supply
- 3 Rushton turbine impellers
- 2 Marine impellers (one scoping, one vortex)
- 4 baffles

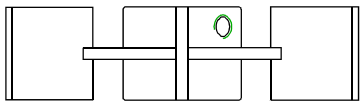
6.1.1 MAGNETIC BOTTOM STIRRER

Z8231B140: Magnetic bottom stirrer assembly for 140L reactor



6.1.2 IMPELLERS AND BAFFLES

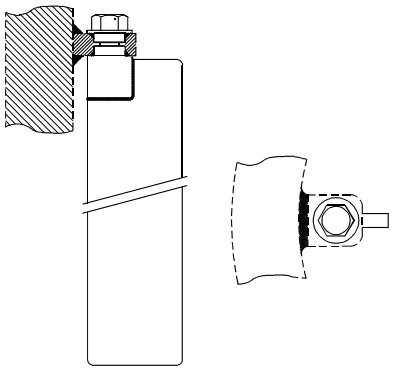
Z82313R650: 6 bladed Rushton (turbine) impeller ID74 OD155



Z82313R651: 6 bladed Rushton (turbine) impeller (×2) ID30 OD155

V6LRL66301: Scoping - Marine Impeller ID30/OD180

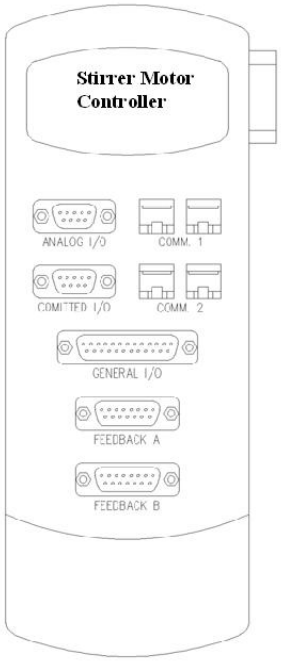
V6LRL66381: Vortex- Marine Impeller ID30/OD180



Z82327K140: Baffles (×4)

6.1.3 MOTOR AND CONTROLLER

V6RRL91203: SERVO Motor + ENCODER 300V, 28.8 Nm



A digital stirrer speed controller (located in the power section) is used to control the stirrer speed

0R0564053: Motor Controller Tuba 20A/230V

For detailed information concerning the controller and stirrer motor, refer to the corresponding Technical Documentation binder or search at http://www.elmomc.com/.

WARNING

- The stirrer motor is a machine with potential hazard (moving parts)
- In order to avoid dangerous situations, the stirrer motor should only be operated when properly installed in the stirrer assembly of the 140L pilot system
- The stirrer motor must only be used as part of the 140L Bioprocess with the control system.

6.1.4 MAINTENANCE

The stirrer motor is designed for low maintenance and can be operated for up to 20, 000 hours maintenance-free. For maintenance instructions, contact the Service department of Applikon Biotechnology.

CAUTION

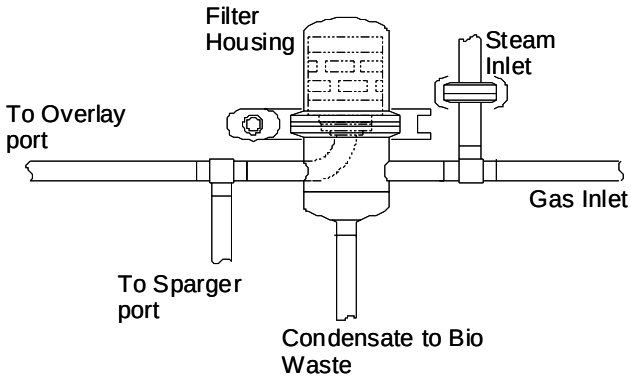
- When carbon brushes are replaced, it is advised also to replace the ball bearings of the motor.
- Operating the stirrer motor with worn-out carbon brushes will cause irreversible damage!
- Installation, maintenance and repair of the stirrer motor shall be carried out only by qualified personnel

6.2 AERATION

This section describes all reactor auxiliaries for gas supply (inlet and outlet).

6.2.1 GAS INLET FILTER ASSEMBLY

The inlet filter assembly is used for aseptical supply of gasses to the bioreactor. It contains a gas filter system (stainless steel housing with a PTFE gas filter cartridge inside).

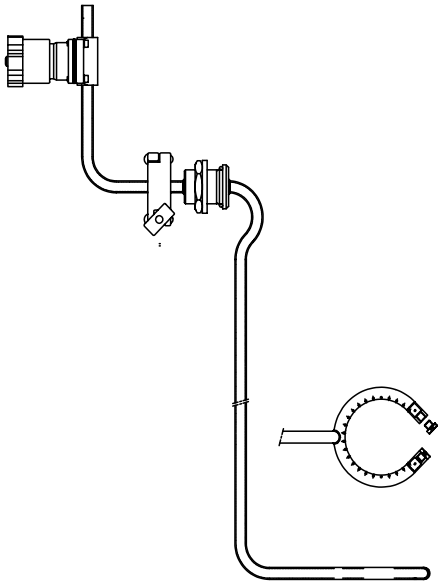


V6KV431001 Automatically operated Inlet filter assembly

6.2.2 SPARGERS AND OVERLAYS

Spargers are used to sparge gas in the medium. The air overlay assembly is used for headspace aeration.

The ring is placed around the shaft of a bottom drive stirrer assembly. The 24 holes (Ø1.5 mm) in the ring are made in such a way that the gas is injected into the medium in the direction of the stirrer shaft.

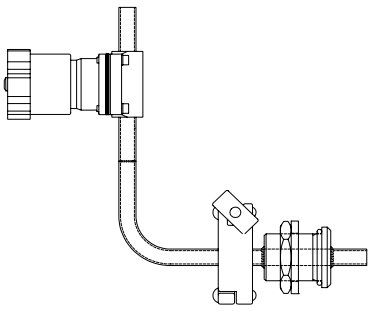


The two ends of the sparger tube are blinded.
A drain hole (Ø1.5 mm) is located at the bottom side of each end.

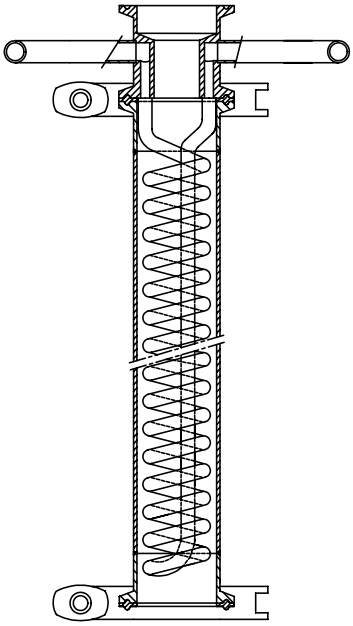
Z82318R140 Ring Sparger

The overlay assembly is used for headspace aeration
It is mounted in the 27 mm port at the upper side wall of the 140L bioreactor.

V6KR733001 overlay inlet for 27 mm port



6.2.3 GAS OUTLET SYSTEM



The air outlet system is used for off gas. It contains:

- An air outlet condenser.
- A steam heater
- A filter.
- A manually operated block valve

Condenser

Z82308C061 Spiral condenser jacketed 3.0" TC

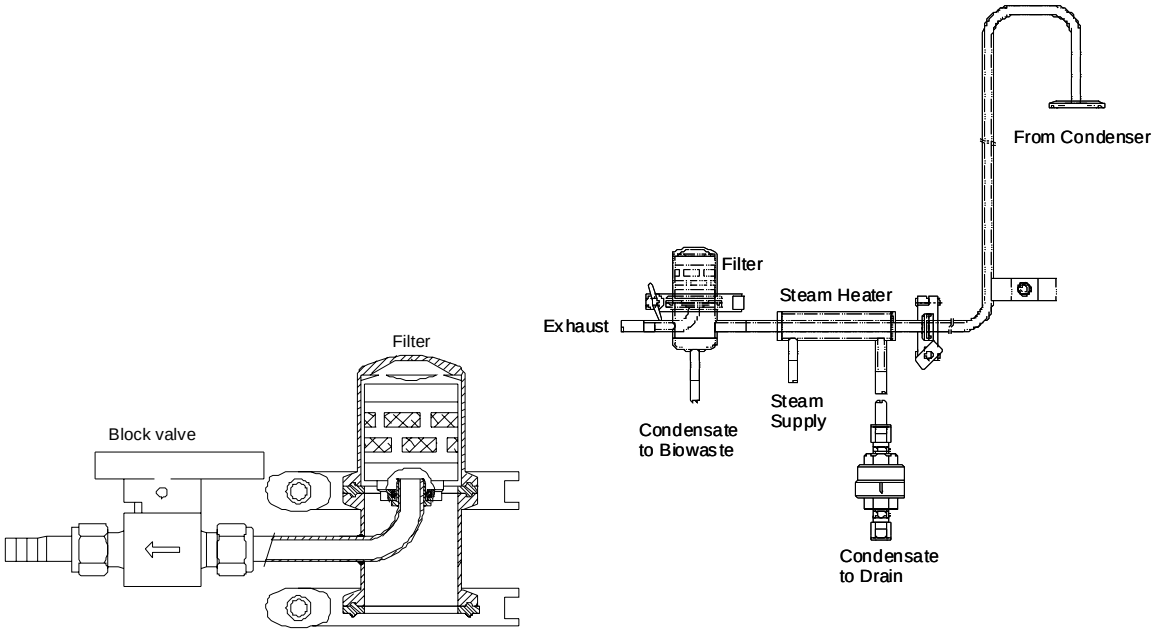
A steam heater

The steam heat exchanger heats up the gas exiting the condenser and thus prevents excessive condensation on the outlet filter

Filter

The filter is needed to prevent back growth contamination and contamination of the environment.

Z82308F010 Exhaust filter 3/4" TC
VOM5221233 Gas outlet manually operated block valve



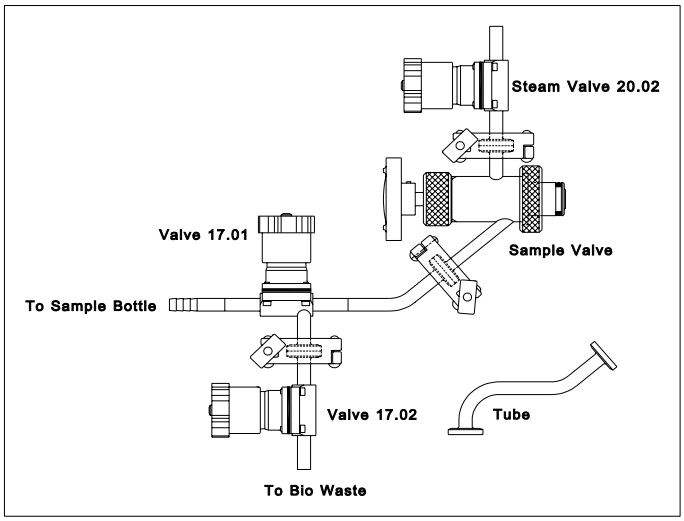
6.3 SAMPLE VALVE AND DRAIN

Samples can be taken from the bioreactor in two different ways:
- using the Applikon Sampling System
- using the Applikon Bottom Drain

During sterilization, but also during cultivation, both systems can be sterilized In Situ (and re-sterilized after sampling).

6.3.1 SAMPLING SYSTEM

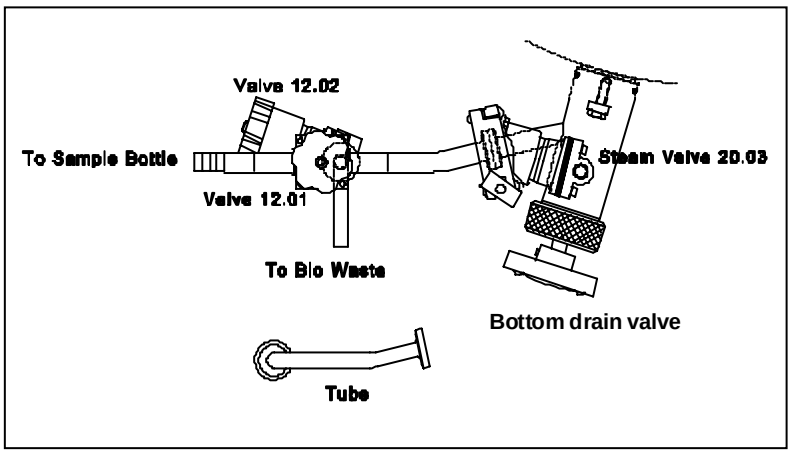
The contained sampling system consists of:
1. a sample valve (Tri-Clamp connection)
2. two additional valves (V-17.01 and V-17.02).



V6LN330711 Contained Sampling System maximum particle size = 1 mm sidewall DN 25 port

6.3.2 CONTAINED BOTTOM DRAIN

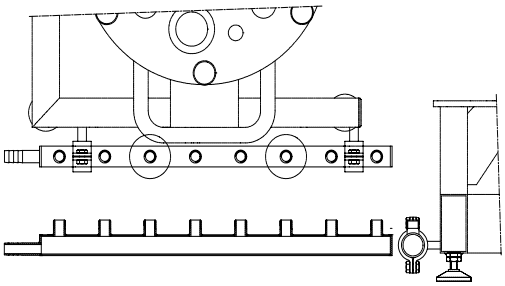
The contained bottom drain consists of:
- a drain valve (Tri-Clamp connection)
- two additional valves (V-12.01 and V-12.02).



The contained bottom drain for the 140L bioreactor is welded on the vessel
V6LF230181 Contained bottom drain maximum particle size = 2 mm

6.3.3 BIO WASTE

The Bio Waste line is a manifold for condensate from the gas inlet filter, sample valve, bottom valve, off gas filter, etc.
Any unused connections are plugged.
Each condensate connection must contain a thermostatic steam trap.

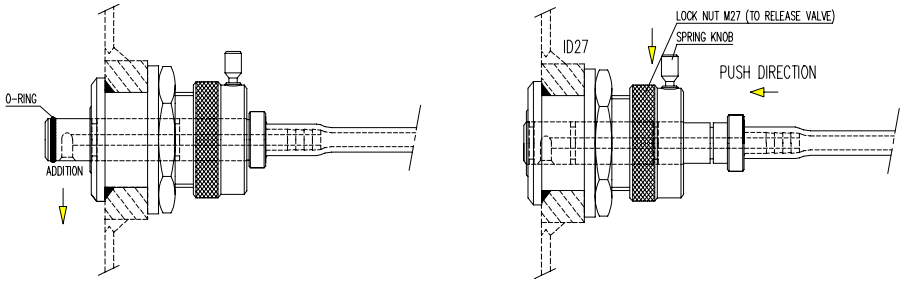


V6KV432101 Industrial Steam Condensate Skid-Mounted
V0WAATRUD3 Thermostatic steam trap

6.4 ADDITION

6.4.1 PUSH VALVE ASSEMBLY

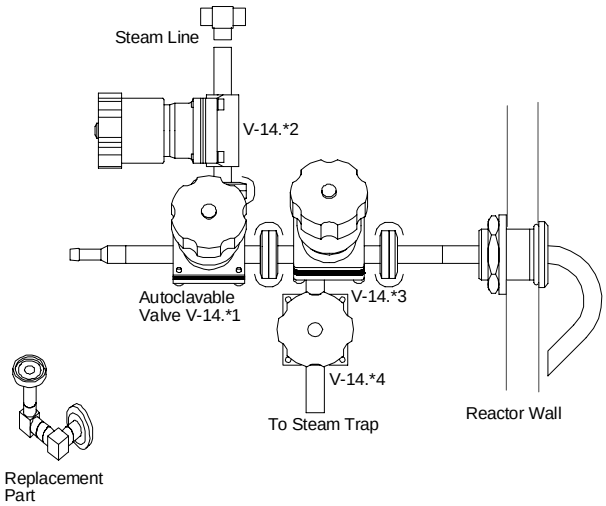
The push valve assembly is designed for easy, aseptical through 27 mm ports in the top plate and the upper sidewall section of the 140L bioreactor. (See the ***i***-Control Operator Manual, section Cultivation – Push-valves).



Z82324MT27 Push valve ID = 3 mm OD = 6 mm 27 mm port
Z82324MT33 Adapter push valve 27mm port

6.4.2 CONTAINED ADDITION ASSEMBLY

The contained addition assembly supports aerosol-free operation and is used for addition of liquids and for inoculation. The assembly is In Situ sterilizable. It contains four (manually operated) membrane valves. During sterilization the inlet part is replaced by the bent tube. The inlet part is sterilized in an autoclave separately.



The inlet connection is Ø 6.35 mm.

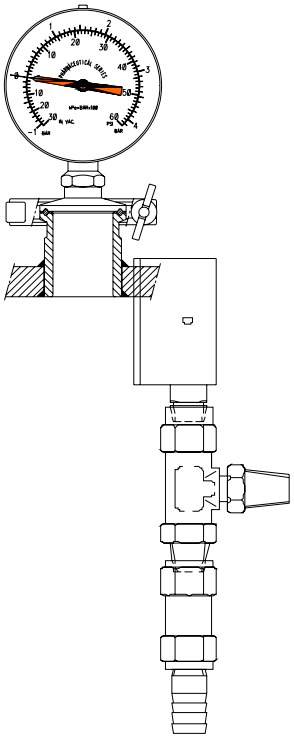
The 140L bioreactor has two contained addition assemblies mounted in Ø 27 mm ports (N24 and N25) at the upper part of the sidewall of the reactor.

Z82324MT21 Contained addition assembly 27 mm port

6.5 SAFETY DEVICES

6.5.1 PRESSURE GAUGE

Z71206PI10 Pressure gauge -1 to 4 barg, -14 to 60 psig 1.5" TC port

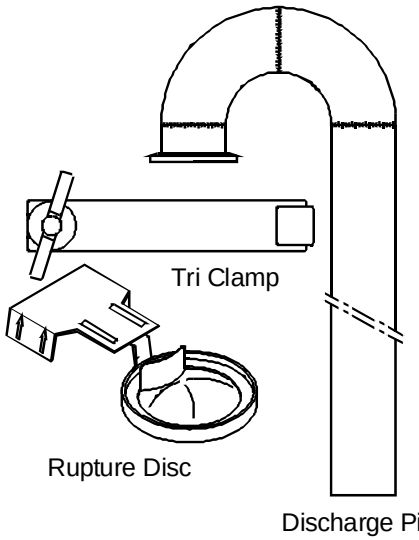


6.5.2 PRESSURE GAUGE AND RELIEF VALVE FOR JACKET

Z71206PR21 Pressure gauge and relief valve for jacket -1 to 5 barg

6.5.3 RUPTURE DISK AND DISCHARGE PIPE

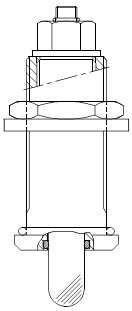
The rupture disks will relieve the pressure at 2 barg.
The rupture disc (together with the discharge pipe) fits on a 1.5" TC port.



Z821302070	Rupture disc	1.5" TC	ASME	2 barg
Z811302085	Discharge pipe	1.5" TC		

6.6 OTHER AUXILIARIES

6.6.1 ILLUMINATION



The illumination assembly is used to illuminate the inside of the bioreactor, allowing the contents to be viewed through the sight glass at the front side of the 140L bioreactor.

The illumination assembly contains a 12 V / 10 Watt halogen bulb and an illumination holder, which is mounted at the head plate.

Z821320090 Illumination ass. 27 mm port

6.6.2 BLIND STOPPERS

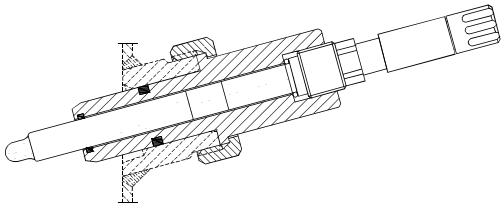
Refer to general arrangement drawing of the 140L bioreactor for blind stoppers and other auxiliaries

7 SENSORS

Sensors for measurement of process parameters are mounted in the head plate or in the lower sidewall ports.

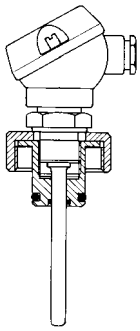
7.1 pH

The gel-filled sensor hardly needs maintenance (except proper cleaning after cultivation).
It fits directly into a sensor holder for a lower DN 25 port.



Z001012051	Sensor	pH+	
Z100500040	Cable	pH sensor	L = 5 m
Z101002512	Sensor Holder	EASYFIX	DN 25 port

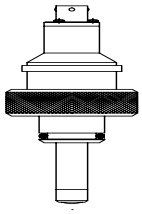
7.2 TEMPERATURE



Z722200002	Sensor Pt-100	Temperature	DN 25 port
Z710402120	Cable	Temp. sensor	L = 3.6 m

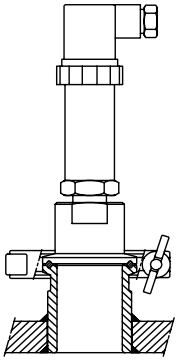
7.3 DO

This dissolved oxygen sensor operates on a polarographic principle.
This sensor fits directly into a lower DN 25 port.
For specific information concerning the sensor, (including information regarding maintenance and the detachable sensor cable), refer to the sensor's user manual (supplied with the sensor).



Z010007025	Sensor, In Situ	DO	L = 70 mm	DN 25 port
Z110500040	Cable	DO sensor	L = 5 m	

7.4 FOAM



Z72205AF20	Sensor	Level	16 mm port
Z710402140	Cable	Level sensor L = 4.1m	

7.5 PRESSURE

The pressure sensor includes the cable.

Z71206PS10	Sensor	Pressure	-1 to 3 bar
Z71501PS10	Sensor	Cable	

EU DECLARATION OF CONFORMITY

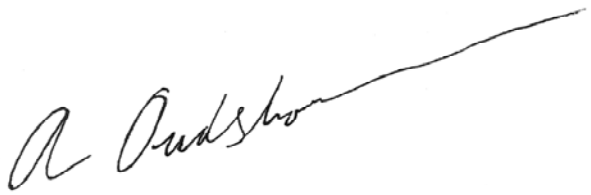
CE

The company Applikon Biotechnology B.V., residing in Schiedam, The Netherlands, hereby certifies that the instrument:

i-Control$_{XP}$ for In Situ sterilizable Bioreactors

meets the requirements of the following EU Directives:

Directive 73/23/EEC on Low Voltage,
Directive 89/336/EEC on Electromagnetic Compatibility; amendment 93/68/EEC and Directive 98/37/EEC on Machinery.



ir A. Oudshoorn MBA, Director September, 28th 2005

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